Table 10

Summary of the analysis of segregants by transduction test, lysate test and by crossing test

Recip. Culture	Trnsd. Lysate	1 Trnsd.	Classification of segregant by Lysate 2	Cross 3 Idiotypic (+)	Idiotypic Tot. Prot.	Allotypic (+)	Allotypic Tot. Prot.
Gal_2^- Lp^s	wild	(1) Gal_2^-	Gal_2^-	0	7805	-	-
		(2) "	"	0	4992	-	-
		(3) "	"	0	106	-	-
		(4) "	"	0	4552	-	-
Gal_2^- Lp^+	wild	(1) Gal_2^-	Gal_2^-	0	4070	-	-
		(2) "	"	0	5384	-	-
		(3) "	"	0	2072	-	-
		(4) "	"	0	6988	-	-
Gal_4^- Lp^s	wild	(1) Gal_4^-	Gal_4^-	0	896	-	-
		(2) "	"	0	918	-	-
		(3) "	"	0	1134	-	-
		(4) "	"	0	863	-	-
Gal_4^- Lp^s	Gal_2^-	(1) Gal_4^-	Gal_4^-	0	2786	3	3183
		(2) "	"	0	2675	2	3471
		(3) "	"	0	3485	23	5342
		(4) "	"	0	5952	1	1665
		(5) "	"	0	5000	1	891
		(1) Gal_2^-	Gal_2^-	7	3102	0	1988
		(2) "	"	10	4364	0	1187
Gal_4^- Lp^+	Gal_2^-	(1) Gal_4^-	Gal_4^-	0	16104	3	1389
		(2) "	"	0	5730	1	164
		(3) "	"	0	3358	0	202
		(4) "	"	0	12848	1	171
		(1) Gal_2^-	Gal_2^-	1	11200	0	827
		(2) "	"	6	10608	0	718
		(3) "	"	3	5000	0	409

1. Test of the segregant against the lysates of a known cultures
2. Test of lysate of the segregant against known cultures
3. Test crossing with know cultures

Table 12

The examination of segregants by testing with lysates of known cultures

Recipient cells	Lp genotype	Trnsd. lysate	Segregants idiotypic	allotypic	amphitypic	total
Gal_1-	s	wild	9	0	0	9
	+		33	0	0	33
Gal_2-	s		16	0	0	16
	+ (1)		20	0	0	20
	+ (2)		15	0	0	15
Gal_4-	s		~~46~~ 31	0	0	~~46~~ 31
	+		20	0	0	20
Gal_1-	s	Gal_2- (3)	6	1	0	7
		(4)	1	0	0	1
		Gal_4-	1	0	0	1
	+	Gal_2- (5)	36	6	0	42
		(6)	18	3	0	21
Gal_2-	s	Gal_1-	20	0	0	20
		Gal_4-	21	1	1	23
	+ (7)	Gal_1-	19	2	0	21
	(8)		14	3	2	19
	(9)	Gal_4-	22	1	0	23
	(10)		9	7	0	16
Gal_4-	~~s~~	Gal_2- (11)	17	2	0	19
		(12)	~~35~~ 18	~~5~~ 3	~~2~~ 0	~~43~~ 21
	+	(13)	16	3	0	19
	r	(14)	15	3	0	18

(1),(8),(10), cultures of W2175. (2),(7),(9), cultures of W1210 (3),(6),(11), lysates of W1210. (4),(5),(12),(13),(14) lysates of W902. W902 is the Lp_2^r parent of W2175.

Table 12

Segregants in table 11 whose classification was confirmed by the action of their lysates on known cultures

Recipient cells	Lp genotype	Trnsd. lysate	Segregants idiotypic	allotypic	total
Gal_1-	+	wild	5	0	5
Gal_2-	+ (1)		4	0	4
	(2)		4	0	4
Gal_4-	s		4	0	4
	+		4	0	4
Gal_1	+	Gal_2- (3)	4	5	9
		(4)	0	3	3
Gal_2-	s	Gal_4-	0	1	1
	+ (5)	Gal_1-	0	2	2
	(6)	Gal_4-	4	0	4
	(7)		0	1	1
Gal_4-	s	Gal_2- (8)	16	3	19
		(9)	0	1	1
	+	(10)	15	3	18
			60	19	79

(1),(5),(6), cultures of W2175. (2),(7), cultures of W1210
(3),(8),(10), lysates of W902. (4),(9), lysates of W1210

Table 18.

Galactose negative cultures giving HFT lysates

HFT culture	Recipient cell	Trnsd. lysate	Nature of Gal^+ reversions	HFT segregant	Nature of Gal+ reversion HFT seg.
Gal_1^-	Gal_1^-	Gal_2^-	unstable	Gal_1^-	stable
	Gal_1^- Gal_2^-,	Gal_1^- + Gal_2^-*	unstable	Gal_1^-, Gal_2^-, Gal_1^- Gal_2^-	stable
Gal_2^-	Gal_2^-	Gal_1^-	unstable	Gal_2^-	stable
	Gal_1^-	Gal_2^-	unstable	$Gal_1^-Gal_2^-$	none observed
	Gal_2^-	Gal_2^-	unstable	$Gal_1^-Gal_2^-$	none observed
	Gal_1^-	Gal_2^-	unstable	Gal_2^-	stable
	Gal_1^-	Gal_2^-	unstable	Gal_2^-	stable
	Gal_2^-	~~Gal_2^-~~*	unstable	Gal_2^-	-
	Gal_4^-	**	unstable	Gal_2^-	stable
	~~Gal_4^-~~	~~Gal_2^-~~			
Gal_4^-	~~Gal_4^-~~	~~Gal_2^-~~	——	——	——
	Gal_2^-	Gal_4^-	not done	Gal_4^-	stable

* Transduction made with a mixture of HFT Gal_1^- and Gal_2^- lysates.
** These lysates were from a mixture of cultures.

Table 14

Correlation of lysogenicity with transduction using lysates giving a high frequency of transduction

1. The transductions

Cells Exposed to	Post Exposure cell titer	Number of colonies observed		
		Gal(-)	Gal(+)	Gal(-)partially lysed
Broth	4.1×10^9	3280	0	0
HFTlysate*	3.5×10^9	2801	31	54

2. Examination of the colonies after HFT lysate exposure

Colony type	Number of colonies examined	Number of colonies		
		Lp^s	Lp^+	Lp^r
Gal(-)	31	31	0	0
Gal(+)	26	0	23	3

* Lambda plaque titer was 1.2×10^9. One ml of cell suspension was added to one ml of lysate and the mixture incubated at 37C for 10 minutes. The cells were then centrifuged down, the supernatant discarded and the cells resuspended in one ml broth. The suspension was then diluted and plated on EMB galactose medium.

Table 16

The [redacted] interaction between Gal_1- and Gal_4-

1. The transductions

Recipient Lp^s cells	Trnsd. HFT lysate	Number of colonies Gal(+)	Gal(-)	Gal(-) papillating
Gal_1-	broth	0	465	0
	Gal_4-	0	316	2
Gal_4-	broth	0	440	0
	Gal_1-	0	408	2

2. Examination of galactose negative segregants derived from galactose positive clones found in papillating galactose negative colonies

Recipient Lp^s cells	Trnsd. HFT lysate	Classification of segregants Gal_1-	Gal_4-	Gal_1-Gal_4-	Gal(-) papillating
Gal_1-	Gal_4-	10	2	0	1
Gal_4-	Gal_1-	5	6	2	4

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151

283-1 x 1476 dem[illegible]

HFJ6, 7⁻ → x 518

Position Effect

I Gal1 - Gal4

	Genotype	Ref.	Seg.
(A)	$\frac{1^- 4^+}{1^+ 4^-}$	Thesis	–
(B)	$\frac{1^+ 4^-}{1^- 4^+}$	Thesis	–
(C)	$\frac{1^- 4^-}{+ +}$	283, 305, 312	
(D)	$\frac{1^+ 4^+ 2^-}{- - 2^+}$	295	135 [illegible] 14 allo 3 [illegible] (1⁻)

II Gal1 - Gal7

	Genotype	Ref.	Seg.
(A)	$\frac{7^+ 1^+}{7^+ 1^-}$	302, 307	7 (7⁻) 4 (1⁻) 3 (1⁻ 7⁻)
(B)	$\frac{7^+ 1^-}{7^- 1^+}$	(323)	
(C)	$\frac{1^- 7^-}{+ +}$	(320)	
(D)	$\frac{1^+ 7^+ 2^-}{- - +}$	(315A)	

11 Genotype Ref Seq

(A) $\frac{5-1+}{6+1-}$ 308

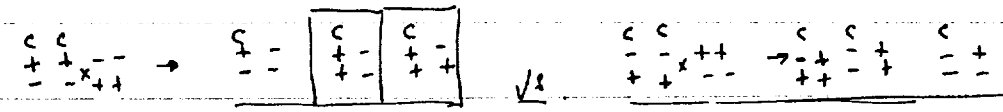
(B) $\frac{6+1-}{6-1+}$ 323

(C) $\frac{6-1-}{+1}$ 320

(D) $\frac{6+1+2-}{6-1-2+}$ 324

Position Effects.

1. Theoretical



2. Evidence – Segregants from single nuclei.

Idio	Allotype	Stbl.	Unstbl	Idio (Segregants)	Allo (Segregants)	Amphi (Segregants)	P.E. (–)	
7⁻	1⁻	4	17	7	4	3	3	c 7 1
1⁻	7⁻	19	4	1	3	0	0	c 7 1
6⁻	1⁻	5	19	8	2	3	6	c 6 1
1⁻	6⁻	16	6	2	4	0	0	c 6 1
4⁻	1⁻	[illegible]	18	10	1	2	[illegible]	c 4 1 (2 not line)
1⁻	4⁻	13	9	6	1	0	2	c 4 1 (reservations because Lp^x)

tentative order: c — 4, 6, 7, — 1

Completion of 7⁻ x 1⁻

			I	A	Amphi
1.	1⁻ 7⁻ —x 8⁻	33/0	29	1	0
2	1⁻ 7⁻ x— (+)	29½/0	24	0	0

Completion 6⁻ x—x 1⁻

			I	A	Amphi	
1.	1⁻ 6⁻ —x 8⁻	5/0	12	4	0	
2.	1⁻ 6⁻ x— (+)	278/0	20	0	0	(3 stable)

Completion 4⁻ x—x 1⁻

				I	A	Amphi
1.	1⁻ 4⁻ —x 8⁻	81/6	71/3	135	14	3
2.	(+) —x 1⁻ 4⁻	c 1000/0		24	0	0

Interactions between 4, 6, 7,

307A 8H f— 7⁻ from cross streak

7 pap → 3(7⁻), 4 (4⁻)

rpt. 3 (7⁻) 3 (4⁻)

310 HFT 6⁻ —x 7⁻ 30/7 (old 6 soln) 27 pbl +

		Sdbl	Unstbl	Idio	Allo	Amph	TE.	Total		
343	HFT 6⁻ —x 7⁻	17	4	0	3 (4)	1(2)	0	4		C 6 7
344	HFT 7⁻ —x 6⁻	7	13	5	0	2	4	11	(2 lost)	
	+ —x 6⁻ 7⁻	–	–	15	0	0	0	15		
	6 7⁻ —x 2⁻	–	–	18	2	1(?)	0	21		

345	HFT 4⁻ —x 6⁻	3	14	14	0	0	0	14		C 7 4
	HFT 6⁻ —x 4⁻	17	6	3 (8)	1 (5)	1 (3)	0	5	(1 lost)	
	+ —x 4⁻ 6⁻	–	–	16	0	0	0	16		
	4⁻ 6⁻ —x 2⁻									

1⁻2⁻ ⟵ 4⁻, Positive effect between 1, 4⁻

Order	C	C	C	C	C	C
	4+	2-	1	1 ①	4	2
	2-	1-	4	2	1	4
	1-	4+ ①	2	4 ②	2	1
	90% (2-)	50% (1-2-)	100% (1-4-)	30% (1-2-) ③	50% (---)	90% (2-)
	5% (---)	50% (---)		30% (---) ②	50% (4-)	5% (1-4-)
	5% (4-)			30% (1-4-) ①		5% (4-)
				10% (2-) ①		

↑ ↑

C 2⁻4⁻ ⟵ 1⁻

30% (1-4-)	90% (2-)	50% (---)	90% (2-) ②	90% (1-4-)	50% (2-4-)
30% (2-4-)	5% (1-4-)	50% (1-)	5% (1-) ①,②	10% (2-)	50% (---)
30% (---)	5% (1-)		2.5% (---) ①		
10% (2-)			2.5% (1-4-) ②		

↑ ↑

Recent studies of recombination in E. coli (17) have led to the discovery of a compatibility mechanism (15), a lysogenic system subject to genetic control (10), and a system of limited transduction by temperate phage (22) comparable to that of Salmonella (28). These three phenomena involve transfer of heritable factors by infection in contrast to bacterial mating which involves the entire genotype. The clarification, differentiation, and interrelationships of these mechanisms were emphasized in this investigation.

I The LYSOGENIC SYSTEM IN E. COLI K-12

The relationship of a temperate phage, λ, to a specific locus, Lp_1 (latent phage) has already been reported (10). In summary, the principal reaction types of bacterial strains are: sensitive (Lp^S), lysogenic (Lp^+), and the non-lysogenic resistant type, Immune-I (Lp^r). In crosses they behave as a system of multiple alleles, linked most closely with Gal_4. This linkage has been confirmed in a Gal^+ Lp^+ x Gal^- Lp^S cross in another laboratory (27). In addition, the two factors segregated out of heterozygous diploids in the parental coupling. This evidence points, therefore, to a genic determinant regulating the maintenance of λ provirus.

From a number of direct and indirect experiments it is known that all these types adsorb λ. A second locus, Lp_2, controls resistance or sensitivity to λ-2, a virulent λ mutant, and is situated in the Mal_1--S region of the chromosome. As Lp_2^r strains cannot adsorb λ, they are therefore not subject to any consequences whose initial reaction requires adsorption; Lp_2 does not interfere with the maintenance of λ previously established in Lp^+ strains. The genotype $Lp^sLp_2^r$ is consequently indistinguishable from $Lp^rLp_2^s$ types with respect to lytic effect of λ. Cross-reactions of λ with λ-2 antiserum have been observed.

New Data on Immune-1: The status of the various isolates of immune-1 strains has been reported, and the interpretation of their constitution with respect to prophage had been reserved pending evidence of a "cryptolysogenic" phage that normally fails to mature to give rise to lytic virus. The segregation pattern of $Gal^+Lp^+/Gal_4^-Lp^r$ diploids, also heterozygous for Mtl and Mal_1 (table 7) is identical with similar Lp^+/Lp^s results. The hypothesis that Lp^r types may carry a non-reproducing prophage is supported by experiments in which a low titer of λ was recovered by U-V induction of at least one (22). Lp^r types are also subject to transduction, and the results of these studies will be deferred to that section.

Incidental Variant Types: No new evidence bearing on the problem on the "semilysogenic" strain (10) can be presented. Tests to determine whether host-modified λ was carried (section III) were negative.

An intermediate host reaction, semiresistant to both λ and λ-2, comparable to the one in Shigella paradysenteriae (26) and the V_1^p allele of K-12 (11) has been clarified. Standard λ suspensions have a reduced efficiency of plating (eop) on this mutant such that the plaques produced are reduced in size and number, and also show a reduced efficiency of transduction. The mutants have been successfully lysogenized, but are still semiresistant to λ-2. The protocols for crosses which establish a mutation at a new Lp_3 locus not linked to Lp_2-Mal or Lp_1 - Gal, and conferring partial resistance to λ, are presented in table 13.

Mechanism of infection; Mutation and Selection vs. Induction: Breeding experiments and diploid segregations reveal only the chromosomal determinant of lysogenicity. The facility of the change Lp^s to Lp^+ encourages the possibility that λ directly induces (rather than selects) Lp^+ among the numerous survivors of exposure to phage. The following types of evidence would be useful in elucidating the primary infection process: (1) identification of a "prelysogenic" genotype in the absence of phage

would encourage the mutation hypothesis. It would be characterized as an apparent immune-1 that would be converted to a stable lysogenic after treatment with λ. (2) a careful study of the dynamics of infection, including the isolation of clonal pedigrees of single cells exposed to λ which engender lysogenics. A pure lysogenic pedigree would favor the induction hypothesis.

Attempts to identify the prelysogenic genotype in K-12, and hybrids of K-12 and other crossable lines have been unsuccessful. Preliminary experiments of the infection process (10) have disclosed lysogenic colonies contaminated with sensitive cells and free phage long after initial contact with λ. These mixed clones have since been confirmed in K-12 (18) and Salmonella (14,21,23B). The possibility that spontaneous alteration of the bacteria predisposing to a lysogenic decision plays some role in the recovery of lysogenics is thus not yet excluded. However, the simplest conception remains that the genetic elements of the phage are directly incorporated in, or attached to the bacterial chromosome as we have been able to find no indication of an extra-nuclear inheritance of lysogenicity.

The Effect of λ and F on Crossing Behavior: The presence of λ in one, both, or neither of the parents of a cross does not influence the yield of recombinants. As noted earlier (8) sensitives were not eliminated

as lethal phenotypes, but the progeny of lysogenic x sensitive included both parental types, and no others, in ratios dependent on the selected auxotroph markers. On the other hand, the compatibility factor (F) determines not only the yield but also the segregation pattern of many overtly unselected markers. Prototrophs are recovered only when at least one parent is F^+; F also seems to direct the elimination of certain chromosomal segments after the formation of the hybrid zygote (15,23). The important distinctions of F and λ are summarized in table 1. These are emphasized to mitigate any confusion that might arise from the suggestions that have been recorded elsewhere that λ may play a direct role in sexual recombination as well as to emphasize the distinction between the λ controlled transduction of restricted genetic factors and the F-controlled sexual recombination. The independent transmission of these factors was demonstrated by the recovery of (1) F^+Lp^S cells on the one hand, and F^-Lp^+ on the other, from mixtures of genetically labelled F^-Lp^S and F^+Lp^+, and similarly, (2) Lp^+F^- (but no Lp^SF^+ or Lp^+F^+) as survivors from F^-Lp^S exposed to λ-containing filtrates from F^+Lp^+ cultures.

II TRANSDUCTION

Cell-free filtrates derived from suitable Salmonella strains were capable of transferring unit genetic factors to a competent recipient (28). A wide range of independent markers has been equally subject to transduction. Additional analysis has shown that the temperate phage of the donor strain is the vector of the genetic material (16,25). Attempts to detect transduction in K-12 among the survivors in the turbid centers of λ plaques were negative (10); but by using high-titer lysates obtained by U-V induction (20), a successful transduction was achieved (22). Two striking contrasts with the Salmonella system were demonstrated: (1) the restriction to a single genetic character, galactose fermentation, and (2) a striking instability manifested by mosaic Gal^+/Gal^- colonies after transduction despite repeated single colony purification on EMB galactose agar. The incidence of persistent instability, rarely if ever encountered in Salmonella (14), varies with the recipient strain.

Confounding of Transduction with Recombination ?: The conditions required for transduction are generally precluded in crossing experiments. Moreover, the unstable mosaic Gal^+/Gal^- colony characteristic of transduction has not been so far recovered among recombinant progeny. A

more careful inquiry into the effect of λ and Gal segregation was necessary, however, in view of the transduction phenomenon, since it may provide an alternative interpretation of the Gal-Lp cosegregation ratios currently satisfied by a linkage explanation. Crosses of genetically related parents differing only in the presence or absence of λ were therefore studied. Table 2 demonstrates no significant deviation in the yield of Gal^+ recombinants where parents vary only for the Lp marker.

Is Transduction a Selection Artefact?: Interaction of genetic factors on reverse mutation of entirely independent loci have been reported before (15). An analysis of the Gal- segregation from the unstable transduction, the allelic transduction, reported below, as well as many other types of evidence (22) rule out the interpretation that the transduction is a selection artefact. The most convincing evidence, however, has been the development of specific Gal^- transductions in Gal^+ recipient strains by means of λ with extraordinary high frequency of transduction (22), when the λ donor was Gal^-.

Transduction and F-transfer: Just as lysogenization is independent of the conversion of F^- into F^+ strains, the transduction mediated by λ is unrelated to the F status of either the recipient or the donor cells.

Crosses of F^- x F^- by standard techniques are completely sterile. However, recombination of two nonallelic Gal^- mutants can be indirectly demonstrated by transduction. Lysates from $Lp^+Gal^+F^-$ were completely functional in introducing the Gal^+ factor to Gal^-F^- cells. Similarly, nonallelism of two Gal^-F^- strains can be established by the formation of Gal^+ in transduction experiments whereas the sexual sterility of the cross would block cell recombination in toto.

Crosses of a strain characterized by its enhanced fertility, Hfr, (15) displayed a linkage of the Hfr trait to Gal (12). These data were verified (table 3) for Gal^-_2. Despite this linkage, efforts to transport the Hfr and Gal^+ factors simultaneously into $Gal^-F^-Lp^S$ recipient cells via λ prepared from Hfr bacteria were unsuccessful. The conversion of F^- to F^+ by λ filtrates from F^+ strains was examined by crossing the Gal^+ transduction with F^- tester strains and was likewise unsuccessful. The competence of λ in transduction therefore continues to be confined to the Gal cluster.

The Concurrence of Transduction and Lysogenization: Observations on the E. coli system, as in Salmonella, are consistent with the hypothesis that the vector of transduction consists of temperate phage. As a rule,

(169)

the transductions isolated from Gal⁻Lp^S bacteria exposed to λ are consistently pure, stable lysogenics, despite the persistent instability of the Gal$^+$ trait; the ensuing Gal⁻ segregants are also lysogenic. Lysogenization occurs very much more frequently than transduction, but the correlation of the two remained to be explored as evidence bearing on the hypothesis. In the first experiment (table 4 , part A) transductions were picked as Gal$^+$ papillae and streaked out on EMB galactose agar. A single Gal⁻ (representing non-transinduced cells) and a single Gal$^+$ (the successful transduction) were each tested for lysogenicity on an appropriate Lp^S indicator. In experiment B, marked Gal$^+$Lp^S cells in the approximate proportions expected from transduction were introduced with the Gal⁻ and the mixed culture on EMB galactose plates. With the assumption that both Lp^S strains would adsorb and be equally affected by λ, a disparity in lysogenizations of the two ensuing Gal$^+$ classes was looked for. Whereas all of the transduction Gal$^+$ were lysogenized, only up to 70% of the artifically inserted Gal$^+$ or of the original Gal⁻ had been infected. Both parts of the experiment show a distinct correlation of lysogenization with transduction; the incidence of lysogenization is almost higher in these than in the control bacteria on the same plates.

Segregation of lysogenic sensitive has not so far been observed (up to 500 tests) from these simultaneously transduced and lysogenized recipients. This evidence argues that λ is the passive vector of genetic material from its source strain. This material is injected to the bacterium by the phage. In Salmonella the transduced genetic factors seem to undergo an immediate substitution for the homologues in the recipient bacterium, if they are successful at all. In E. coli K-12, however, an intermediate stage is perceived where one can detect simultaneously the presence of the original recipient and the new transduced genetic factors in the same cells by virtue of their subsequent segregation. The relationship between this replacement of genetic material and the conversion of virulent λ into its prophage stage ("reduction" 6) has not yet been completely worked out. As will be described below, however, these processes have been separated and are therefore not mutually dependent.

Lysogenization of Immune-1 in Transduction Experiments: When immune-1 strains such as W-1027 and W-1924 are exposed to λ, no evidence of their lysogenization is ordinarily perceived. However, under conditions where transductions can be selectively isolated about 5% of these altered bacteria

(171)

are also found to have been lysogenized. Repeated serial segregation of the resulting transductions showed that in some cases, lysogenicity failed to segregate. In others, lysogenicity and Gal segregate together, while in a single instance a lysogenic Gal^- segregant was found which continued to segregate Lp^r colonies. Sometimes a very weak lysogenicity is observed ("one-plaque types" in cross-brush tests), which is completely lost after a few transfers. Some of these atypical cases are presented in table 5, and suggest the following alternative interpretations: (1) Lp^r cells are genetically lysogenic but carry a modified prophage. These cells are generally resistant to infection with λ. However, λ may be exceptionally introduced simultaneously with the Gal^+ fragment and there may displace the avirulent form of the prophage, or when Lp segregation is observed, both prophages persist together for the time being. (2) The Lp^r is a "null" allele. In transduction, Lp^+ and Gal^+ factors are introduced, but the lysogenic/immune segregation occurs when Gal segregates. This hypothesis can not account easily for the Gal^-Lp^+/r types except by devising a complicated scheme involving crossingover. (3) Immunes may or may not be genetically lysogenic. The production of Lp^+ signifies the occurrence of a double transduction at two loci, Gal and Lp. (a) ordinarily these linked factors would tend

(72)

to be lost as a block in the ensuing segregation, or (b) a linked transduction does not operate. By a two-step process, two effective particles have penetrated; one fragment carries Gal^+, the other Lp^+. Independent segregation is permitted and a mechanism requiring the breakage of a 2-factor linked fragment as in (2) is not called for.

In any event, special assumptions must be made on the avidity of the Lp^s locus for pro-λ to account for the failure of transductions to Lp^s to segregate Lp^+/Lp^s along with Gal^+/Gal^-. However, the Lp^r may only block the propagation of λ or its reduction to pro-λ.

Hypothesis (1) accounts for the occurrence of immunes which can be induced by U-V (22). The recovery of unstable Lp^+ transductions in non-transinduced Gal- would tend to support hypothesis 3. The most decisive elucidation of whether transduction displaces a mutant phage particle with a wild type λ or whether a normal Lp^+ allele is substituted for a mutant or null host Lp^r gene would be provided by experiments with genetically distinguishable λ preparations. Lp^r/Lp^s transductions were prominent with irradiated λ, tending to support hypothesis 2.

Irradiation effects: Quantitative assays of transducing potentiality of phage preparation are necessarily based on plaque counts. The survival

after various treatments of plaque-producing particles and transducing particles are not identical either in Salmonella (28) or K-12 (22). In fact, it is known from both studies that transducing power may be increased at some intermediate dosages. A comparison of the effects of U-V and X-radiation is given in table 6. A U-V dose reducing plaque assay from $1/2 \times 10^{10}$ to 16.9×10^{5} per ml yielded 170 transductions from an initial titer of 10^{3} / ml. A comparable X-ray dose was found to be between 150,000 and 200,000 r. No recognizable transductions were recovered at the latter exposure. Two viewpoints are indicated: (1) the lytic and transducing principles in λ are separable by their independent survival, and (2) avirulent λ particles are produced but they are damaged only to the extent of virulence for the host cell. Conclusive evidence favoring one or the other views of Lp^{r}, however, is not yet at hand. A decisive chemical and genetic separation of the transducing material from the virus particle has not yet been experimentally achieved, whether or not it is at all theoretically possible.

GENETIC DEFINITION OF THE GAL LOCI

Recombination: Attention was focused on galactose nonfermenting mutants because of the coincidence of the first recognized λ-sensitive

mutant in Gal^-_4 (W-518), and the subsequent observation of linked segregation of Lp and Gal_4 (10). Gal^- mutants have been isolated directly by inspection of surviving colonies after U-V treatment on EMB galactose agar and also as non-papillating variants of Lac^- mutabile recovered on EMB lactose agar plates. Interaction of Gal^- and Gal^+ on the phenotypic expression and reverse mutation of Lac_1 and Lac_7 alleles have been described (9). Recombination analysis provided the evidence for a cluster of four linked Gal loci (7). Gal_1 and Gal_4 show a very low order of crossovers. Preliminary data could only differentiate them on the basis of behavior in Het crosses; Lp and Gal_1 are both hemizygous, while Gal_4^+/Gal_4^- heterozygous diploids are readily obtained (table 7).

Transduction: Transduction tests reinforce standard allelism tests (table 8), and in fact have tentatively identified several new loci, now awaiting confirmation by recombination analysis. Whether the relative yield of Gal^+ transductions is proportional to the map distance between Lp and the Gal locus is in question. The results of large-scale allelism tests made available to date by new techniques to facilitate crossing are summarized in table 9.

(75)

The instability characteristic of the Gal^+ transduction results in the mosaic colony already noted and deserves further comment. Despite passage through a large number of serial single colonies, Gal- segregants are almost always thrown off. In transductions from Gal^+, i.e. $Gal^+ \rightarrow Gal^-$, these Gal^- segregants have been identified as alleles of the locus of the original recipient strain, both by crossing and further transduction tests. No other kinds of Gal^- have been recovered. On the other hand, if the donor is a non-allelic Gal^-, both donor and recipient Gal^- appear among the segregants from the Gal^+ transduction (22). For example, $Gal_2^- \rightarrow Gal_4^-$ gives galactose-fermenting intermediates, presumably of the constitution $Gal_2^- Gal_4^+ / Gal_2^+ Gal_4^-$. The segregants in all these tests are identified by (1) crossing experiments with Gal_2^- and Gal_4^- testers, (2) deriving λ and subjecting the testers to its action, and (3) applying λ from Gal^+, Gal_2^-, Gal_4^-, etc. The $Gal_2^- Gal_4^-$, a crossover type, has not been conclusively and consistently established. This double mutant would be identified as one which is subject to transduction by λ from Gal^+ and from any Gal^- other than Gal_2^- or Gal_4^-, and would yield no Gal^+ recombinants in crosses with Gal_2^- and Gal_4^- testers.